

Application No.: 09/437,726
Page 11

APPENDIX B

CLAIMS PENDING IN USSN 09/437,726 WITH ENTRY OF THIS AMENDMENT

27. A method for obtaining an isolated polynucleotide comprising a sequence encoding a protein having Rubisco carboxylation activity, the method comprising:

recombining a plurality of parental polynucleotide species encoding at least one protein having Rubisco carboxylation activity under conditions suitable for sequence shuffling to form a resultant library of sequence-shuffled polynucleotides;

transferring said library into a plurality of host cells, thereby forming a library of transformants wherein sequence-shuffled Rubisco polynucleotides are expressed;

identifying at least one transformant from said library that expresses a protein having a Rubisco carboxylation activity that is significantly enhanced relative to the Rubisco carboxylation activity of proteins encoded by the plurality of parental polynucleotide species, wherein the identified transformant contains a polynucleotide comprising a sequence encoding the protein having an enhanced Rubisco carboxylation activity; thereby obtaining a polynucleotide comprising a sequence encoding the protein having an enhanced Rubisco carboxylation activity.

28. (amended) The method of claim 27, wherein the encoded protein having an enhanced Rubisco carboxylation activity has a higher carboxylation specificity factor than proteins encoded by the plurality of polynucleotide species.

29. (amended) The method of claim 27, wherein the encoded protein having an enhanced Rubisco carboxylation activity has a velocity of carboxylation that is greater than that of proteins encoded by the plurality of polynucleotide species.

30. (amended) The method of claim 27, wherein the encoded protein having an enhanced Rubisco carboxylation activity has a velocity of oxygenation that is less than that of proteins encoded by the plurality of polynucleotide species.

31. (amended) The method of claim 27, wherein the encoded protein having an enhanced Rubisco carboxylation activity has a K_m for CO_2 that is less than that of proteins encoded by the plurality of polynucleotide species.

32. (amended) The method of claim 27, wherein the encoded protein having an enhanced Rubisco carboxylation activity has a K_m for O_2 that is greater than that of proteins encoded by the plurality of polynucleotide species.

Application No.: 09/437,726

Page 12

33. (amended) The method of claim 27, wherein the plurality of parental polynucleotide species encodes at least one Rubisco Form I L subunit.
34. (amended) The method of claim 27, wherein the plurality of parental polynucleotide species encodes at least one Rubisco Form I S subunit.
35. (amended) The method of claim 27, wherein the plurality of parental polynucleotide species encodes at least one Rubisco Form II subunit.
36. (amended) The method of claim 27 further comprising a selectable marker gene which affords a means of selection when expressed in chloroplasts.
37. (amended) The method of claim 36, wherein the sequence encoding a protein having Rubisco carboxylation activity and the selectable marker gene are flanked by an upstream flanking recombinogenic sequence having sufficient sequence identity to a chloroplast genome sequence to mediate efficient recombination and a downstream flanking recombinogenic sequence having sufficient sequence identity to a chloroplast genome sequence to mediate efficient recombination.